

(11) Publication number:

**0 109 942**  
**A2**

(12)

**EUROPEAN PATENT APPLICATION**

(21) Application number: 83850273.0

(51) Int. Cl.<sup>3</sup>: **A 61 K 39/00**  
**C 07 G 7/00**

(22) Date of filing: 13.10.83

(30) Priority: 18.10.82 SE 8205892

(43) Date of publication of application:  
30.05.84 Bulletin 84/22(84) Designated Contracting States:  
AT BE CH DE FR GB IT LI LU NL SE(71) Applicant: Morein, Bror  
Krokusvägen 14  
S-175 74 Järfälla(SE)(72) Inventor: Morein, Bror  
Krokusvägen 14  
S-175 74 Järfälla(SE)(74) Representative: Fagerlin, Helène et al,  
H. Albinns Patentbyrå AB Box 7664  
S-103 94 Stockholm(SE)

(54) Immunogenic protein or peptide complex, method of producing said complex and the use thereof as an immune stimulant and as a vaccine.

(57) The invention relates to an immunogenic complex containing antigenic membrane proteins or peptides from viruses mycoplasmas, bacteria, parasites or animal cells or prepared synthetically or with hybride DNA technique and glycoside. The invention also relates to a process for producing the complex, whereby microorganisms, animal cells, proteins and peptides are mixed with solubilizing agents in buffered, possibly saline, solution, whereby complexes are formed between charged monomeric antigenic proteins and detergent or peptides and solubilizing agents, whereafter the charged monomeric antigenic proteins or peptides are separated from solubilizing agents in the presence of, or are separated from solubilizing agent and directly transferred to, a glycoside solution which contains one or more glycosides with hydrophobic and hydrophilic regions in a concentration of at least the critical micellar concentration, whereby a protein complex is formed, which is isolated and purified. The invention also relates to the use of the immunogenic complex as an immunity-stimulating agent, especially as a vaccine, and compositions containing the complex.

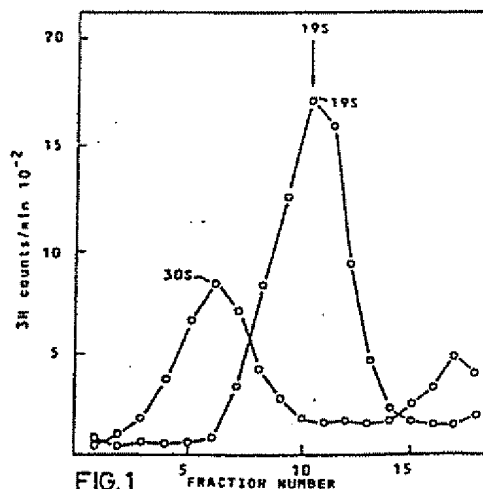


FIG.1

The glycoside can be any glycoside at all with hydrophobic and hydrophilic regions. Preferably, the saponins are used which are described in R Tschesche and Wulf, Chemie und Biologie der Saponine in Fortschritte der Chemie Organischer Naturstoffe, published by W Herz, H Grisebach, G W Kirby, Vol 30 (1973), especially the strongly polar saponins, primarily the polar triterpensaponins such as the polar acidic bisdesmosides, e.g. saponin extract from Quillajabark Araloside A, Chikusetsusaponin IV, Calendula-Glycoside C, Chikusetsusaponin V, Achyranthes-Saponin B, Calendula-Glycoside A, Araloside B, Araloside C, Putranjia-Saponin III, Bersamasaponoside, Putranjia-Saponin IV, Trichoside A, Trichoside B, Saponaside A, Trichoside C, Gypsoside, Nutanoside, Dianthoside C, Saponaside D, preferably aescine from Aesculus hippocastanum (T Patt and W Winkler: Das therapeutisch wirksame Prinzip der Rosskastanie (Aesculus hippocastanum), Arzneimittelforschung 10(4), 273-275 (1960) or sapoalbin from Gypsophilla struthium (R Vochten, P Joos and R Ruyssen: Physico-chemical properties of sapoalbin and their relation to the foam stability, J Pharm Belg 42, 213-226 (1968), especially saponin extract from Quillaja saponaria Molina, primarily the DQ-extract which is produced according to K Dalsgaard: Saponin Adjuvants, Bull Off Int Epiz 77 (7-8), 1289-1295 (1972) and Quil A which is produced according to K Dalsgaard: Saponin Adjuvants III, Archiv für die Gesamte Virusforschung 44, 243-254 (1974). Also mixtures of glycosides can be used. The amount of glycoside added should be at least 1-3 times their critical micelle formation concentration (CMC), preferably at least 5, especially at least 7-12 times. It is assumed that the glycoside in this case can be bound to and catch monomer forms of the membrane proteins. Preferably Quil A is used, which has a critical micelle formation concentration of 0.03% by weight. The amount of Quil A should then be at least 0.02% by weight, especially 0.05-0.5% by weight, preferably 0.2% by weight. The citations above concerning the